

Selected Topics: Toxicology

BIPHASIC RATTLESNAKE VENOM-INDUCED THROMBOCYTOPENIA

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Abstract—Thrombocytopenia is a common occurrence in moderate to severe crotaline envenomation. The exact mechanism by which rattlesnake venom leads to thrombocytopenia is unclear, but aggressive treatment with crotaline-specific antivenom often leads to resolution of this disorder. Crotalinae Polyvalent Immune Fab (CroFabTM, Protherics Inc., Nashville, TN) (crotaline Fab) is now available for the treatment of symptomatic rattlesnake envenomation. Although recurrence of thrombocytopenia has been reported in patients after envenomation treated with crotaline Fab, cases refractory to this therapy have not been described. We report a case of severe crotaline envenomation that appears to have exhibited two separate episodes of thrombocytopenia, only one of which responded to antivenom. The second, later phase was refractory to both crotaline Fab as well as traditional Antivenin (Crotalinae) Polyvalent (Wyeth-Ayerst Pharmaceuticals, Philadelphia, PA) (ACP). By reviewing the literature regarding venom-induced thrombocytopenia, we attempt to explain this “biphasic” phenomenon and the inability of crotaline Fab to reverse this toxic effect. © 2003 Elsevier Science Inc.

Keywords—rattlesnake; crotaline; venom; envenomation; thrombocytopenia

INTRODUCTION

Venom-induced thrombocytopenia (VIT) in victims of rattlesnake envenomation has been reported. In these cases, the presence and severity of thrombocytopenia

seems to depend on the particular venom composition as well as the amount inoculated (1–8). Although the true clinical importance of VIT is unclear, this phenomenon remains of concern to treating physicians as bleeding complications occasionally occur (2,8). Due to its recent release, few data are available regarding the use of crotaline Fab for the treatment of VIT. In the presented case, VIT treated with crotaline Fab exhibited an initial response and subsequent resistance to therapy. We attempt to explain this phenomenon in the context of the presented case and available literature describing VIT.

CASE REPORT

A 42-year-old man presented to the Emergency Department (ED) 30 min after sustaining a rattlesnake strike to the left index finger. The patient kept the involved snake as a pet and was envenomated when he attempted to hand-feed the reptile. He was able to identify the snake as a red diamond rattlesnake (*Crotalus ruber ruber*). This was the first captive snake that the patient had ever owned and he denied any previous snake envenomations. He denied performing any first aid prior to hospital arrival. At the time of presentation, the patient's vital signs were: blood pressure of 157/88 mm Hg, pulse of 86 beats/min, respiratory rate of 16 beats/min, and temperature of 36.1°C (97.0°F). The left index finger was noted to have two visible puncture wounds on the radial aspect

just proximal to the distal interphalangeal joint with blood oozing from each. There was swelling of the entire left hand to the level of the wrist. No systemic manifestations of envenomation were noted, but the patient appeared to be mildly intoxicated. Presentation laboratory values were reported to be normal except for a platelet count of 2000 per mm^3 . He was treated with intravenous fentanyl for analgesia and transferred to our hospital where crotaline Fab was available. ACP was not initially administered due to concerns over allergic reactions.

On arrival at our institution approximately 4 h after his envenomation, he complained of severe left hand and arm pain. Further history revealed no allergies to medications and no contributory past medical conditions. Vital signs and pulse oximetry were normal. Upon physical examination, he had significant swelling of the left fingers, hand, and arm with extension into the axilla. Blood was oozing slowly from the puncture wound sites and two fluid-filled blebs were already present on the palmar surface of the index finger. The patient's left axilla was full and exquisitely tender with ecchymosis extending from the axilla onto the chest wall. There was no pain with passive flexion and extension of any fingers and, while swollen, no forearm compartments appeared to be tense. The finger tips were somewhat dusky but capillary refill was brisk. He continued to demonstrate no systemic signs of envenomation. Admission laboratory values at our institution were remarkable for platelets of 22,000 per mm^3 , fibrinogen of 107 mg/dL, and prothrombin time of 16.4 s (INR = 1.6).

Therapy was initiated immediately with six vials of crotaline Fab. His pain was controlled with morphine sulfate. Laboratory values 1 h after initial antivenom infusion demonstrated an improvement in platelets to 215,000 per mm^3 , fibrinogen to 161 mg/dL, and prothrombin time of 13.5 s (INR = 1.3). A bleeding time measured immediately after antivenom infusion was 5 min and 30 s (normal bleeding time < 7 s). Reevaluation of local symptoms 4 h after antivenom infusion showed progression of arm swelling and bruising of the axilla and chest wall. A second infusion of 4 vials of crotaline Fab was administered.

Repeat laboratory values 1 h after the second dose of antivenin showed platelets of 107,000 per mm^3 , fibrinogen of 191 mg/dL, and prothrombin time of 12.7 s. Increasing bleb formation at the index finger and worsening of swelling and ecchymosis at the axilla and chest wall was noted at this time. Ecchymosis extended from the level of the axilla down below the waistline onto the gluteal region (Figure 1). Limb circumference measurements were unchanged. Another 4 vials of crotaline Fab was infused.

Local symptoms and signs remained unchanged 1 h after the third dose of antivenom. Steady improvement of

local venom effect was noted throughout the rest of the patient's hospitalization. Serial laboratory evaluations over the next 5 days, however, continued to reveal worsening thrombocytopenia, necessitating multiple repeat doses of crotaline Fab administered in 2–4 vial increments (Figure 2). Each subsequent dose of crotaline Fab appeared to have diminishing effects on platelet counts. Bleeding time measurements corresponding to platelet counts of 93,000 per mm^3 and 7,000 per mm^3 were 13 min and > 20 min, respectively. Fibrinogen and prothrombin time, once normalized, remained within normal ranges. On the fourth hospital day, 10 vials of ACP were administered in response to a platelet level of 5000 per mm^3 . This therapy also had no response. A total of 46 vials of crotaline Fab and 10 vials of Wyeth antivenom were administered during the patient's hospital course.

Local wound debridement was carried out over the next several days, with significant tissue loss evident on the left index finger. After platelet count stabilization, the patient was discharged on the ninth hospital day with outpatient hand surgery clinic follow-up. He subsequently underwent a partial left index finger amputation.

Toxicology clinic follow-up on the eleventh day after envenomation revealed significant improvement of arm and hand swelling. Laboratory evaluation at that time showed platelets of 216,000 per mm^3 . No symptoms of serum sickness were reported at the clinic visit or subsequent telephone follow-up.

DISCUSSION

Rattlesnake venom-induced thrombocytopenia (VIT) can occur in the absence of significantly abnormal coagulation studies. Isolated thrombocytopenia has been reported in association with envenomations by the timber rattlesnake (*C. horridus horridus*), tiger rattlesnake (*C. tigris*), southern Pacific rattlesnake (*C. viridis helleri*), northern blacktail rattlesnake (*C. molossus molossus*), and the red diamond rattlesnake (*C. ruber ruber*) (1–7,9,10). Russell suggests that untreated, resolution of VIT usually can be expected within 72 h, but longer durations have been observed (9,10). Successful treatment of VIT with ACP also has been reported and most authorities agree that intravenous antivenom infusion remains the therapy of choice (6,11). In most cases, this therapy causes rapid improvement in platelet counts; however, reports do exist of VIT in which the platelet drop has been resistant to antivenom therapy (1,8,9,12). In at least two reported cases, bleeding has occurred in association with VIT (2,8). Very few data are available regarding VIT treated with crotaline Fab.

Despite well-documented clinical experience and multiple animal studies, the exact pathophysiology of



Figure 1. Extension of venom-induced tissue damage and ecchymosis onto the chest wall.

venom-induced thrombocytopenia is still unclear. Proposed mechanisms include decreased platelet production, aggregation, and sequestration.

Decreased Production

In 1971, Lyons reported a case of rattlesnake envenomation by a red diamond rattlesnake (*C. ruber ruber*) with subsequent VIT (8). This patient demonstrated severe thrombocytopenia without significant hypofibrinogen-

emia. A bone marrow biopsy performed during the course of this patient's thrombocytopenia showed normal marrow with adequate reserves of megakaryocytes. Similar observations of normal bone marrow during VIT have been made by Wingert et al. in rabbits injected with northern Pacific rattlesnake venom and again by Tallon et al. in a patient after timber rattlesnake envenomation (2,13). These observations suggest that VIT is not related to decreased marrow production, but more likely aggregation or peripheral sequestration.

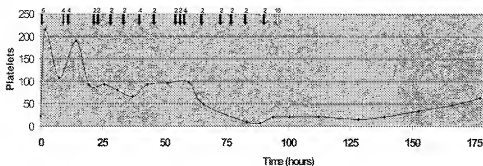


Figure 2. Platelet counts and antivenom vials (during hospital admission).

Aggregation

Schmaier et al. in 1980 reported the identification of a specific serine protease, crotalocytin, isolated from venom of the timber rattlesnake (*C. horridus horridus*). Crotalocytin has been shown to cause platelet aggregation and activation in vitro (14,15). This observation is consistent with past reports of VIT occurring after timber rattlesnake envenomation (1,3,4). Because *C. horridus horridus* venom is not used in the production of Wyeth antivenin, specific antibodies to crotalocytin may not be contained in the Wyeth formulation (1). This lack of antibodies could explain the variable response to ACP observed after envenomation by this particular snake. Hardy et al. also reported the presence of similar platelet aggregating proteins in *C. molossus* venom (7). Because of the heterologous nature of rattlesnake venom, it is highly likely that many similar such proteins are contained in venoms of other species. The presence of platelet aggregating venom constituents may explain the early or acute fall in platelet counts and subsequent early, rapid improvement after antivenom therapy observed in our case and by previous authors.

Sequestration

In 1981 Simon and Grace studied the effects of sublethal doses of *C. atrox* venom in rabbits. These animals were infused with radiolabeled platelets prior to venom injection. Platelet survival studies demonstrated platelet consumption with subsequent resolution, presumably due to increased and ongoing production. In this study, radiolabeled platelets were observed to be sequestered near the venom injection site rather than removed by the reticuloendothelial system. Antivenom therapy in these animals did not affect thrombocytopenia, however, it did improve coagulopathy as demonstrated by normalization in fibrinogen and FDPs (16).

The findings of the above study as well as the known effects of venom on tissue vasculature may help to explain the delayed phase of VIT. Venom proteases cause localized small vessel rupture, subsequently provoking platelet aggregation at the envenomation site. Vessel wall injury allows platelets to adhere to the exposed subendothelial surface and initiate a process of recruitment and aggregation of other platelets. Subjects clinically manifest swelling, petechiae, and purpura at the site of envenomation (5,8,10,17). Platelet sequestration at the site of vascular injury would be expected to produce delayed platelet effects not readily reversed by antivenom. By this mechanism, the severity of tissue injury would determine the degree of late thrombocytopenia. However, the external appearance of the wound may not

always reflect the underlying vascular injury, amount of exposed endothelial surface, and subsequent late thrombocytopenia.

In summary, previous investigations do not suggest decreased platelet production or removal of platelets by the reticuloendothelial system as primary mechanisms for VIT. Evidence suggests that platelet-aggregating venom constituents as well as platelet sequestration in areas of vascular damage near the envenomation site are responsible. Our patient experienced both early and delayed thrombocytopenia after envenomation. Initial doses of crotaline Fab were very effective in normalizing platelet counts, suggesting Fab binding to platelet aggregating venom proteins and rapid subsequent release of these platelets. Coincident with the stabilization of local venom effects in our patient, platelet counts again began to decrease and were progressively resistant to repeat doses of crotaline Fab. This late finding likely represented sequestration of platelets at the envenomation site onto exposed, damaged endothelium. The severity and refractoriness of our patient's delayed VIT was most likely the result of his significant local tissue injury. It appears that VIT in our patient was *biphasic*, the early phase representing binding of platelets by venom proteins and later caused by sequestration at the envenomation site.

Recurrence of envenomation symptoms 24 to 48 h after crotaline Fab therapy has been reported. This phenomenon has been previously explained as the rapid clearance of Fab antivenom in the face of a depot site of venom (18–20). Generally, these patients respond promptly to repeat antivenom dosing (20,21). The severity and refractory nature of our patient's VIT are more difficult to attribute to this pharmacokinetic explanation.

The clinical relevance of VIT is unknown and has never been formally tested. Only two case reports exist of bleeding associated with VIT (2,8). Hematemesis, gross hematuria, and gingival bleeding were reported in one patient, and rectal bleeding in another. In our patient, bleeding times were checked after early crotaline Fab therapy, during thrombocytopenia, and after platelet normalization. Bleeding times were found to be highly abnormal during periods of thrombocytopenia and normal after early antivenom administration and platelet normalization. These measurements imply that severe VIT may represent a bleeding risk.

In this case antivenom therapy was withheld in order to transfer the patient to a facility in which crotaline Fab antivenom was available. Concerns regarding allergic reactions to ACP led to this transfer and caused a delay in treatment of approximately 4 h. The decision to give antivenom therapy for rattlesnake envenomation is always based on a risk vs. benefit analysis. However, it is important to remember that the availability of a new

antivenom formulation does not change the indications for therapy. In cases where antivenom is clearly indicated, the treating physician may need to initiate therapy regardless of the formulation available. The administration of ACP does not preclude the use of crotaline Fab later.

CONCLUSION

Venom-induced thrombocytopenia refractory to antivenom therapy has been reported. The mechanisms responsible for this phenomenon are not well understood. In the presented case, biphasic thrombocytopenia was observed with the delayed phase being refractory to crotaline Fab therapy. We postulate that early VIT is precipitated by platelet aggregating proteins contained in venom and later may recur due to local venom effects at the wound site.

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